LETTERS TO THE EDITOR

Scope

Heart welcomes letters commenting on papers published in the journal in the previous six months. Topics not related to papers published earlier in the journal may be introduced as a letter: letters reporting original data may be sent for peer review.

Presentation

Letters should be:

- not more than 600 words and six references in length
- typed in double spacing (fax copies and paper copy only)
- signed by all authors

They may contain short tables or a small figure. **Please send a copy of your letter on disk.** Full instructions to authors appear in the July 1999 issue of *Heart* (page 116).

Of bombers, radiologists, and cardiologists: time to ROC

EDITOR,—Dr Collinson suggests that it is time that cardiologists use the ROC (receiver operator characteristic) curve and that it "avoids the pitfalls of sensitivity and specificity".[1] While the ROC curve is undoubtedly useful in describing the performance of a test and in comparing tests, I find the claim a little surprising as the ROC curve is simply a series of sensitivities and specificities with the cut off sweeping from minimum sensitivity to minimum specificity.

Second, it is recommended that the "point of maximum curvature" is chosen as the optimum trade off between sensitivity and specificity. This is true if the costs of false positives and false negatives are equal—but only if these are equal, which is by no means always the case. Next the point of maximum curvature needs to be judged: in Collinson's fig 1 (for creatine kinase (CK) isoenzyme MB) the curve turns quite sharply at approximately (0.05, 0.87) and again at (0.17, 0.98) but between the two points the slope is fairly constant. The closest the ROC curve gets to (0, 1; the top left hand corner) is approximately (0.15, 0.95). Depending on the relative importance for clinical decision making of sensitivity and specificity, one could choose between these three points. These then need converting back via table 2 to CKMB cut offs of approximately 12 (sensitivity more important), 16 (sensitivity and specificity of equal importance), and 26 (specificity more important). For myoglobin the range of optima (Collinson's fig 2) is wide, from approximately (0.12, 0.64) at the first shoulder to (0.55, 0.94) at the second.

Finally, as the ROC curve is sensitivity–specificity (or a series of sensitivities and specificities), it is difficult to see how it "minimises the prevalence problem". Sensitivity and specificity are features of a test (and the ROC curve helps in the choice of the cut off) but predictive values (positive and negative) depend on prevalence.

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1 Collinson P. Of bombers, radiologists, and cardiologists: time to ROC. *Heart* 1998;**80**:215–17.

This letter was shown to the authors, who reply as follows:

Dr West has read my article with a distinct lack of enthusiasm for the ROC curve. Clearly he prefers sensitivity and specificity and regards my brief (and illustrative) article as the definitive statement on the subject. This, while flattering, is clearly not the case and deserves some comment.

His opening statement misquotes the last paragraph where I have said "largely avoids the pitfalls of sensitivity and specificity". If Dr West is of the view that a single sensitivity and specificity calculation is better than ROC then I must disagree. ROC is much better than a single sensitivity and specificity calculation, which can be arbitrarily selected to maximise one (apparently) desirable threshold largely for the reasons he illustrates.

With regard to the second paragraph Dr West makes some excellent points, which well illustrate the sensitivity and specificity problem. There is a need for caution in his interpretation of a dataset chosen to illustrate what a ROC curve is and how it is derived. The issue of the "cost" of false positive versus false negatives is of great significance to any clinical diagnostic tests, but in routine clinical practice in real patient groups (as opposed to population based studies) the objective is to maximise both sensitivity and specificity for individual patient diagnosis. The points that he raises are more fully discussed in the excellent review paper by Hendersen.[1]

In respect of his final point I would reiterate the last paragraph of the article, ROC curves are better than single sensitivity–specificity calculations but cannot abolish the prevalence problem. In that I concur with Dr West.

1 Hendersen AR. Assessing test accuracy and its clinical consequences. *Ann Clin Biochem* 1993;**30**:521-39.

Is the Framingham risk function valid for northern European populations?

EDITOR,—Predicting the risk of coronary heart disease will always be prone to error. Haq *et al* compared four different risk functions[1]: the Framingham (USA),[2] PROCAM (prospective cardiovascular Münster, German),[3] Dundee (UK),[4] and British regional heart study (UK) risk[5] functions. These functions were applied to 206 male patients attending the Sheffield hypertension clinic. Haq *et al* used Bland–Altman difference plots to compare methods. Although they claim good agreement among the Framingham, PROCAM, and Dundee functions, close inspection shows that the difference in risk in the Framingham—PROCAM plot greatly increases above a mean coronary heart disease risk of 4% (fig 1B), and points in the Framingham—Dundee plot diverge above 0% mean coronary heart disease risk (fig 2B)—that is, there is poor agreement among the various methods. What is more, Haq *et al* seem to dismiss the British regional heart study function because its estimate of risk was fourfold lower than for the Framingham function yet the British study function was able to predict 59% of major ischaemic heart disease in subjects over the ensuing five years.[5]

Surely it would have been more informative to have applied each of the risk functions to subjects who attended the Sheffield hypertension clinic and who were followed up over five years and to see whether the predictions of risk were accurate. Risk analysis is a tricky business. We should use these functions and tables only if we are aware of their limitations.

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1 Huq IU, Ramsay LE, Yeo WW, *et al.* Is the Framingham risk function valid for northern European populations? A comparison of methods for estimating absolute coronary risk in high risk men. *Heart* 1999;**81**:40–6.
2 Anderson KM, Odell PM, Wilson PWF, *et al.* Cardiovascular disease risk profiles. *Am Heart J* 1991;**121**:293–8.
3 Assman G. *Lipid metabolism disorders and coronary heart disease*. München: MMV-Medizin-Verl, 1993.
4 The Dundee coronary risk-disk for management of change in risk factors. *BMJ* 1991;**303**: 744–7.
5 Shaper AG, Pocock SJ, Phillips AN, *et al.* Identifying men at high risk of heart attacks: strategy for use in general practice. *BMJ* 1986; **293**:474–80.

This letter was shown to the authors, who reply as follows:

We agree that risk prediction is concerned with the probability of a future event and is not an exact science. We have shown reasonable—but by no means perfect—agreement between predictions by the Framingham, Dundee, and PROCAM equations. The important question is whether the agreement is close enough for clinical practice. The analysis shown in fig 5 addressed this point and showed that the Framingham risk function separates clearly groups at high and low risk as determined by the two other risk functions. The accuracy of targeting was acceptable and this supports the use of methods based on the Framingham equation in national and international guidelines.

The British regional heart study function predicted *relative* risk well but seriously underestimated absolute risk compared with the other three risk functions. Possible reasons for this were discussed—for example, inclusion of people with established coronary heart disease, different definitions of risk variables, exclusion of HDL cholesterol, and the lower average risk of the population studied. The predictive value of the British regional heart study risk function that Johnston cites[1] is for an *internal* validation, meaning that the risk function was tested in the population from which it was derived. Any systematic error would be common to the derivation and the testing of the risk function and would not therefore be detected. The British regional heart study risk function appears to have important inaccuracy for absolute risk in two external validations.[2]

It would of course be ideal to carry out a prospective cohort study, but the simpler analysis presented reassures us that use of the Framingham function is reasonable, at least in men. We agree that one must be aware of the limitations of risk functions. Coronary heart disease risk assessment methods based on Framingham are much more accurate than use of cholesterol or lipid thresholds, intuitive estimation of risk, or simple counting of risk factors.

1 Shaper AG, Pocock SJ, Phillips AN, *et al*. Identifying men at high risk of heart attacks: strategy for use in general practice. *BMJ* 1986;**293**:474–80.
2 Thompson SG, Pyke SDM, Wood DA. Using a coronary risk score for screening and intervention in general practice. *Journal of Cardiovascular Risk* 1996;**3**:301–6.

Gene therapy made difficult

EDITOR,—While we found your recent editorial on gene therapy very interesting,[1] some points were raised that invite further comment.

Inflammatory responses seem inevitable following exposure to "first generation" adenovirus vectors; however, transgene selection appears to be an important factor in avoidance of these responses.

Your editorial states " . . .(t)his inflammatory response is . . .generally observed using the sort of adenoviral loads needed to achieve expression of the transgene". Undoubtedly, many early in vivo studies of adenovirus mediated gene therapy required very high virus doses to elicit significant transgene expression and therapeutic effects. However, a number of recent studies have obtained significant results with much lower virus doses. Sata *et al*, using an adenovirus expressing Fas-ligand (a cell surface/secreted protein), achieved a significant reduction in neointima formation with a dose of 1×10^6 plaque forming units (pfu)—approximately 1000-fold lower than doses typically used in trials of cytostatic treatment.[2] Shears *et al* demonstrated reduced neointima formation using an iNOS expressing vector at a similarly low virus dose (2×10^6 pfu).[3] Therefore, in vascular tissues, transgenes giving rise to either a secreted protein or a protein that gives rise to a secreted product seem to afford some advantage, perhaps by requiring infection of only a small percentage of cells in the vessel wall. In both studies, transgene expression was under the control of the cytomegalovirus immediate–early promoter. It is probable that the use of smooth muscle cell specific promoters (in the vascular setting) will allow more efficient transgene expression and therapeutic effects from even lower virus doses with concomitantly reduced inflammatory responses.

As your editorial suggests, injudicious use of non-autologous transgenes may result in transgene induced immune responses. However, both autologous and non-autologous transgenes, which themselves downregulate the host immune responses to vector administration, have been shown to improve substantially transgene expression and persistence.[2 4]

Contrary to Dr Clesham's suggestion, deletion of adenoviral genes from vectors has offered a substantial—if not quite revolutionary—improvement in vector efficiency. Stable transgene expression has been demonstrated in immunocompetent mice 10 months after a single injection of "gutless" adenovirus vector expressing α_1-antitrypsin from genomic DNA.[5] Furthermore, "gutless" vectors with space for the insertion of 30 kb of DNA allow the prospect of efficient transgene expression from genomic DNA and production of vectors containing a variety of transgenes, some of which may be aimed at suppression of the host immune response to the vector.

Finally, while host inflammatory responses have attracted much attention, their practical sequelae are not clearly defined in vascular tissues. Despite evidence suggesting that the inflammatory responses in intact arteries may cause neointimal hyperplasia,[6] all studies of adenovirus mediated vascular gene therapy that have compared "no virus" and "control virus" groups have demonstrated no significant difference. Inflammatory changes are undeniably precipitated by exposure to adenovirus vectors, but they do not appear to be deleterious in the setting of gene therapy for restenosis.

It is wise to exercise caution regarding the prospects of human gene therapy, but the omens are less portentous than Dr Clesham suggests. Many of the technical problems initially encountered have been addressed successfully, while rapid progress is being made in others. Much of the future difficulty for gene therapy lies in determining which genes offer the best prospects as therapeutic agents rather than in struggling to make poorly expressed, pro-inflammatory transgene products fit roles to which they are not suited. While there is little virtue in pressing ahead recklessly with what are still largely experimental treatments, it seems unlikely that we will have to wait 25 years before the first human is successfully treated by direct gene transfer, particularly in the vascular setting.

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1 Clesham GJ. Gene therapy made difficult. *Heart* 1998;**80**:313–14.
2 Sata M, Perlman H, Muruve DA, *et al*. Fas ligand gene transfer to the vessel wall inhibits neointima formation and overrides the adenovirus-mediated T cell response. *Proc Nat Acad Sci* 1998;**95**:1213–17.
3 Shears LL, Kibbe MR, Murdock AD, *et al*. Efficient inhibition of intimal hyperplasia by adenovirus-mediated inducible nitric oxide synthase gene transfer to rats and pigs in vivo. *J Am Coll Surg* 1998;**187**:295–306.
4 Ilan Y, Droguett G, Chowdhury NR, *et al*. Insertion of the adenoviral E3 region into a recombinant viral vector prevents antiviral humoral and cellular immune responses and permits long-term gene expression. *Proc Nat Acad Sci* 1997;**94**:2587–92.
5 Schneider G, Morral N, Parks RJ, *et al*. Genomic DNA transfer with a high-capacity adenovirus vector results in improved in vivo gene expression and decreased gene toxicity. *Nat Genet* 1998;**18**:180–3.
6 Newman KD, Dunn PF, Owens JW, *et al*. Adenovirus-mediated gene transfer into normal rabbit arteries results in prolonged vascular cell activation, inflammation and neointimal hyperplasia. *J Clin Invest* 1996;**96**:2955–65.

This letter was shown to the author, who replies as follows:

Kingston and Heagerty raise a number of important issues in their response to my editorial on gene therapy and I am grateful for their interest.

While the prospects for this emerging technology are unknown, some more definite conclusions can be drawn from the past 10 years. It should be remembered that there is no gene therapy in clinical use at present, despite an almost unprecedented research effort.

One of the underlying aims of the current approach is that the biological effects observed following gene transfer should result from the expressed transgene rather than the vector that delivers it. Unfortunately, acute inflammation at the site of vector delivery is an inevitable, non-specific response to conventional doses of adenoviral vectors. This inflammatory response appears to be independent of the transgene or native adenoviral gene expression as ultraviolet inactivated or defective adenoviral particles can induce inflammation and activate the transcription factor NFkB[1 2] This side effect is particularly unhelpful in the context of arterial gene therapy given the current understanding of atherosclerosis as an inflammatory disorder.[3]

Given the non-specific effects of high adenoviral loads, the ability to use very low adenoviral doses seems attractive and may be possible if more potent promoters are incorporated into gene transfer vectors.[4] Reports describing the use of very low viral loads of cytomegalovirus driven vectors (10^6 pfu) are inconsistent with the findings of the vast majority of researchers in this field. Gene transfer in vivo is an inherently inefficient process; there are few reports of meaningful dose–response curves and even fewer examples of excessive transgene expression.

The immune response to adenovirus mediated gene transfer has been extensively studied and has driven the development of so called "gutless" vectors. These "ultimate" adenoviral vectors have been around for some years[5]; however, I am unaware of any significant impact of these newer adenoviral vectors on the disappointing results of hundreds of human gene therapy protocols over the past decade.

Careful evaluation of the problems of inefficiency, local inflammation, and regulation of gene expression highlight the difficulties in trying to transduce cells in patients. As Kingston and Heagerty point out, the application of gene transfer techniques in complex polygenic disorders is further complicated by uncertainty about which genes to overexpress. We can look to cystic fibrosis, haemophilia, and other diseases with clear molecular targets as barometers of the feasibility of therapeutic overexpression in clinical practice. I for one would be surprised if gene therapy for these conditions becomes established without major advances in the currently available vector systems.

1 McCoy RD, Davidson BL, Roessler BJ, *et al*. Pulmonary inflammation induced by incomplete or inactivated adenoviral particles. *Hum Gene Ther* 1995;**6**:1553–60.
2 Clesham GJ, Adam PJ, Proudfoot D, *et al*. High adenoviral loads stimulate NFkB dependent gene expression i human vascular smooth muscle cells. *Gene Therapy* 1998;**5**:174–80.
3 Ross R. Atherosclerosis- an inflammatory disease. *N Engl J Med* 1999;**340**:115–26.
4 Clesham GJ, Browne H, Efstathiou S, *et al*. Enhancer stimulation unmasks latent gene transfer after adenovirus-mediated gene delivery into human vascular smooth muscle cells. *Circ Res* 1996;**79**:1188–95.
5 Fisher KJ, Choi H, Burda J, *et al*. Recombinant adenovirus deleted of all viral genes for gene therapy of cystic fibrosis. *Virology* 1996;**217**:11–22.